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Host DNA Degradation after Infection of *Escherichia coli* with Bacteriophage T4: Dependence of the Alternate Pathway of Degradation Which Occurs in the Absence of Both T4 Endonuclease II and Nuclear Disruption on T4 Endonuclease IV

KATHLEEN A. PARSON AND D. PETER SNUSTAD*

Department of Genetics and Cell Biology, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108

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Escherichia coli cells infected with T4 phage which are deficient in both nuclear disruption and endonuclease II exhibit a pathway of host DNA degradation which does not occur in cells infected with phage deficient only in endonuclease II. This alternate pathway of host DNA degradation requires T4 endonuclease IV.

Within 2 to 3 min after infection of *Escherichia coli* cells with T-even bacteriophage, the nucleoids of the bacteria undergo "nuclear disruption" during which the host DNA moves from its largely central location in the host cell into tight juxtaposition with the cell membrane (12, 13). Subsequent to nuclear disruption, the majority of the host DNA is degraded to acid-soluble form, and the nucleotides produced are re-incorporated into progeny phage DNA (6, 7, 9, 10, 21). Both of these processes are under the control of the phage genome (see the review by J. F. Koerner, 8). Mutants of phage T4 which are deficient in the ability to induce nuclear disruption (D. P. Snustad and L. M. Conroy, J. Mol. Biol., in press) and which are blocked in the degradation of host DNA (5, 20) have been isolated. Neither process is essential for phage reproduction (5, 20; Snustad and Conroy, J. Mol. Biol., in press).

Bacteriophage T4 DNA contains the base hydroxymethylcytosine rather than cytosine which is present in most DNA including that of *E. coli* (23). This difference appears to explain the ability of phage T4 to induce the degradation of the DNA of its host without self-destruction. Two host DNA-specific deoxyribonucleases which are induced after infection of *E. coli* with T4 phage have been purified and characterized (14, 15). One, T4 endonuclease II, induces single-strand breaks in double-stranded DNA (14). The other, T4 endonuclease IV, induces single-strand breaks in exposed single-stranded regions (gaps) of double-stranded DNA (15).

Mutants of phage T4 which are deficient in the ability to induce endonuclease II (17) are blocked at an early stage in host DNA degradation (5, 20). T4 mutants which fail to induce the synthesis of endonuclease IV have also been isolated (2, 16, 19; E. M. Kutter, personal communication). The role of endonuclease IV is less clear, however, since endonuclease IV-deficient mutants exhibit at most a minor block in the degradation of host DNA (2, 19). Amber (*am*) and temperature-sensitive mutants in genes 46 and 47 of phage T4 are also blocked in the degradation of host DNA, accumulating host DNA fragments of size 10^6 to 10^7 daltons under restrictive conditions (11, 22). The exact functions of the gene 46 and gene 47 products have not been identified; thus, it is not clear whether these genes play a direct or indirect role in host DNA degradation.

It had been assumed (see, for example, ref. 18, p. 370) that nuclear disruption was involved in the process of degradation of host DNA. We have recently shown, however, that the degradation of host DNA occurs at the same rate in the absence of nuclear disruption as it does in its presence, providing that endonuclease II is present (D. P. Snustad, K. A. Parson, H. R. Warner, D. J. Tutus, J. M. Wehner, and J. F. Koerner, J. Mol. Biol., in press). Surprisingly, T4 mutants which are deficient in both nuclear disruption and endonuclease II induce a pathway of degradation of host DNA which is not evident in cells infected with T4 mutants deficient only in endonuclease II (Snustad et al., J. Mol. Biol., in press; see Fig. 2). The absence of nuclear disruption thus appears to open or enhance an alternate pathway of host DNA degradation bypassing the endonuclease II block imposed by *den* A mutants. In this

paper, we present the results of studies which indicate that this alternate pathway of host DNA degradation is dependent on the presence of T4 endonuclease IV.

In all of the experiments reported here, *am*N82 (gene 44) has been used to block phage DNA synthesis and thus prevent re-incorporation of host DNA breakdown products into phage DNA. The complete genotypes of the T4 strains used in this study are summarized in Fig. 1.

We have previously reported (Snustad et al., J. Mol. Biol., in press) that the slow pathway of degradation of host DNA which occurs in cells infected with *am*N82-*ndd*98-*nd*28 × 6 (*ndd*98 is a nuclear disruption-deficient mutation in gene D2b; *nd*28 × 6 is an endonuclease II-deficient mutation in gene *den* A) does not occur in cells infected with *am*N82-*r*PT8-*nd*28 × 6 (see Fig. 2). The nuclear disruption-deficient deletion mutant *r*PT8 is missing all or part of genes *r*IIA, *r*IIB, D1, D2a, and D2b (1, 3, 4). Since endonuclease IV is absent in cells infected with T4 mutants defective in the D2a region (16, 19; E. M. Kutter, personal communication), it seems likely that the absence of this alternate pathway of degradation of host DNA in cells infected with *am*N82-*r*PT8-*nd*28 × 6 is due to the absence of endonuclease IV. This possibility has been investigated using quadruple mutants in which *am*N82, the endonuclease II-deficient mutation *nd*28 × 6, and nuclear disruption-deficient mutation *ndd*98 are combined with *r*II deletions extending to various distances into the D region (Fig. 1). The results (Fig. 2) strongly support the hypothesis that endonuclease IV is required for this alternate pathway of host DNA degradation. The T4 quadruple mutant containing deletion A105, which terminates in the D1 region and is thus D2a$^+$, induces the same rate of degradation of host DNA as does the triple mutant *am*N82-*ndd*98-*nd*28 × 6. The quadruple mutant containing deletion *r*H23, which extends into the D2a region, induces very little degradation of host DNA, like *am*N82-*r*PT8-*nd*28 × 6. Thus, the slow pathway of host DNA degradation which occurs in the absence of both endonuclease II and nuclear disruption either (i) requires endonuclease IV or (ii) is an epistatic effect requiring the presence of endonuclease IV and the absence of the *r*IIA, *r*IIB, or D1 gene product or some combination thereof. The simplest interpretation of these results is that, during this slow process of host DNA degradation in the absence of endonuclease II, endonuclease IV is required to convert the limited number of single-strand breaks (and putative single-strand gaps) present in the host

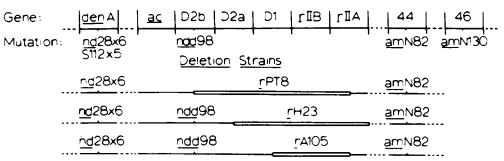

FIG. 1. *Genetic composition of the T4 strains used. Deletion mutants rH23 and rA105 as well as amber mutants N82 and N130 were obtained from R. S. Edgar via H. Bernstein. Deletion rPT8 was generously provided by S. Champe. The isolation and characterization of nd28 × 6, S112 × 5, and ndd98 have previously been described (20, 5; and Snustad and Conroy, J. Mol. Biol., in press, respectively). Multiple mutants were synthesized and identified using previously described procedures (Snustad and Conroy, J. Mol. Biol., in press; 20). The point mutations have arbitrarily been placed at the midpoints of their respective genes. In most cases, their intracistronic location is not known. The relative positions of ndd98 and the D2b end point of the nuclear disruption-deficient deletion rPT8 are also unknown.*

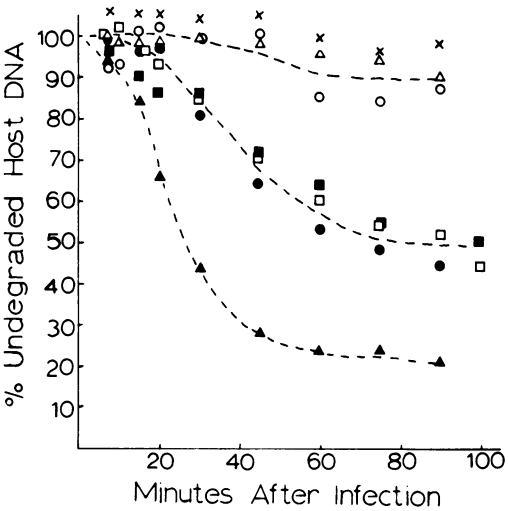

FIG. 2. *Degradation of host DNA to acid-soluble form after T4 phage infection. E. coli strain B/5 DNA was prelabeled with [^{14}C]thymidine (specific activity = 33 μCi/μmol; final concentration = 0.05 μCi/ml) and the conversion of the labeled DNA to acid-soluble products at various times after infection was determined using the previously described procedures (Snustad et al., J. Mol. Biol., in press) except that the multiplicity of infection used in these experiments was five. Incubation was at 30 C. Cells were infected with amN82 (▲), amN82-nd28 × 6, (×) amN82-rPT8-nd28 × 6 (△), amN82-ndd98-nd28 × 6 (□), am N82-ndd98 × 3-S112 × 5 (■), am N82-rH23-ndd98-nd 28 × 6 (○), and amN82-rA105-ndd98-nd28 × 6 (●).*

DNA (Snustad et al., J. Mol. Biol. in press) to double-strand breaks before any extensive degradation to acid-soluble products can occur.

Whether the D2b (*ndd*) gene product is itself a nuclease or whether the effect of nuclear disruption on this alternate pathway of host DNA degradation is indirect, for example, simply a compartmentalization effect, is unknown. The host DNA may simply be more accessible to endonuclease activity when it is distributed throughout the cytoplasm than it is when present in close juxtaposition with the cell membrane (Fig. 3).

We have attempted to determine whether a host endonuclease or residual T4 endonuclease II activity due to in vivo leakiness of the *nd*28 × 6 mutation is responsible for the first stage (presumably the induction of single-strand breaks) in this alternate pathway of host DNA degradation. The same rate of host DNA degradation occurs when the *den* A mutation S112 × 5 (5; generously provided by J. S. Wiberg) is used in place of *nd*28 × 6 (Fig. 2). Thus, if residual T4 endonuclease II is involved, both *nd*28 × 6 and S112 × 5 must be leaky in vivo. We have also observed the same alternate pathway of host DNA degradation in the endonuclease I-deficient host, *E. coli* strain B/41

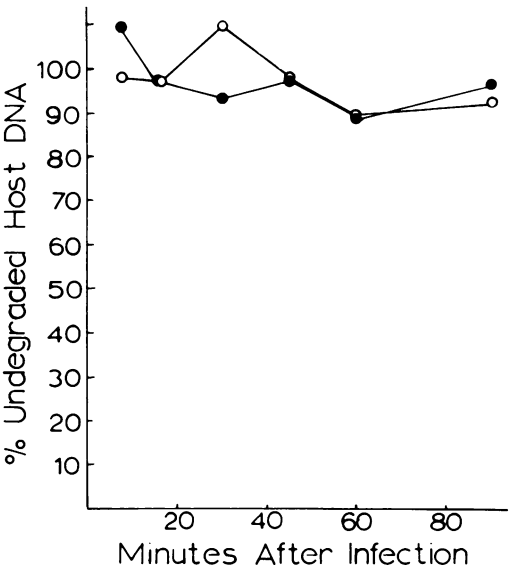

FIG. 4. *Degradation of host DNA to acid-soluble form after T4 phage infection. The procedures are as described in the legend to Fig. 2.* (●) *amN82-amN130;* (○) *amN82-amN130-ndd98* × *2.*

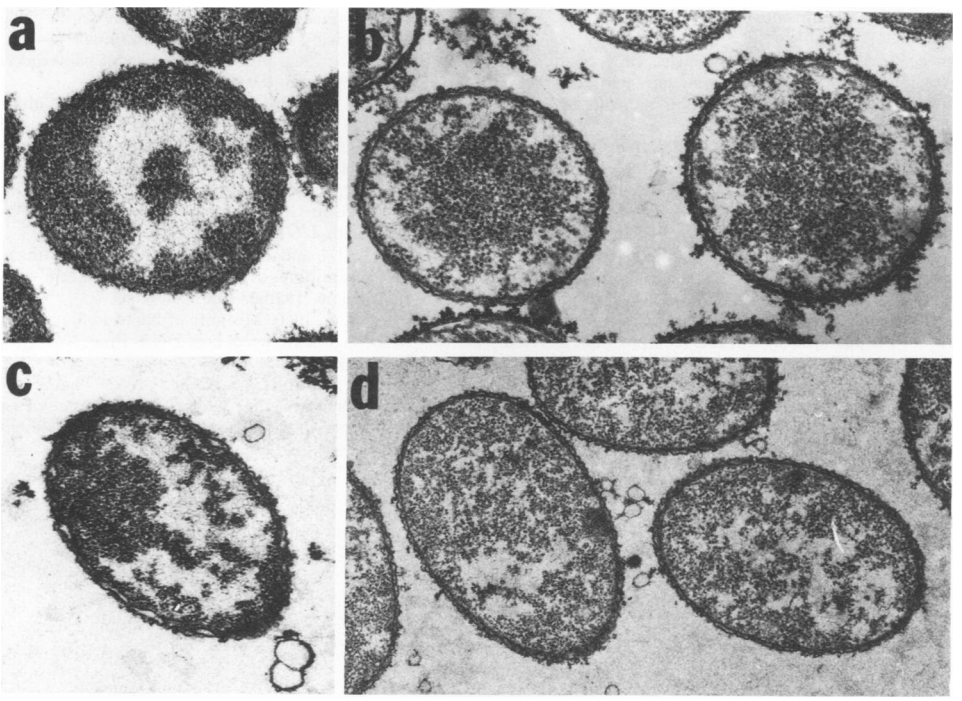

FIG. 3. *Electron micrographs showing the intracellular location of host DNA in an uninfected B/5 cell* (a) *and in B/5 cells infected with endonuclease II-deficient T4 phage which are either nuclear disruption proficient* (b) *or deficient* (c) *and* (d). *Under the conditions of staining used, the DNA-containing regions appear light and the ribosomes and membranes are dark. The procedures used have been described in detail elsewhere (Snustad and Conroy, J. Mol. Biol., in press). Representative cross-sections are shown of* (a) *an uninfected B/5 cell,* (b) *cells fixed at 10 min after infection with amN82-nd28* × *6,* (c) *and* (d) *cells fixed at 10 and 60 min, respectively, after infection with amN82-ndd98-nd28* × *6. Magnification ranges from* ×*20,000 to* ×*30,000.*

(generously provided by E. M. Kutter), and in a *Rec* A$^-$B$^-$ host (kindly provided by J. F. Zissler) (data not shown). It is not clear, therefore, whether residual T4 endonuclease II activity or host nuclease activity (or both) is responsible for the first step of this alternate pathway of host DNA degradation.

Whatever the pathway by which the absence of nuclear disruption allows the degradation of host DNA to bypass the block normally imposed by *nd*28 × 6 or S112 × 5 (endonuclease II-deficient), this pathway does not provide a bypass around the block imposed by mutants in gene 46. No degradation of host DNA to acid-soluble form is observed in cells infected with *am*N82-*am*N130 (gene 46)-*ndd*98 (Fig. 4).

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